the coexpressed GPCRs EP2 or EP4 results in clearly distinguishable effects even though both receptors activate the Gs-cAMP signaling pathway. The mechanism regulating these distinct signaling outcomes remains unclear. Here we show that EP4, in contrast to EP2, dynamically localize in elongated areas of the plasma membrane that are dictated by the underlying cortical microtubule network. For EP4, this results in efficient but transient cAMP production throughout a range of PGE2 concentrations. In contrast, EP2 induces marginal but continuous cAMP levels. We propose a model where, differently from actin corrals and transient confienemtn zones, cortical microtubules form scaffolds for signaling hubs visited by receptor diffusing within the plasma membrane. This leads to efficient regulation of this GPCR signaling function, which is important to regulate cytoskeleton remodeling and migration in leukocytes and cancer cells.

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Nanoarchitecture of Integrin Receptor Clusters on Very Soft Substrates

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Sensing of extracellular matrix (ECM) physical properties is critical for several processes such as cell differentiation, cell migration in the developmental (haptotaxis) and disease (cancer metastasis). Clustering of matrix-activated integrins is an important step in the formation of matrix adhesions and clustering can occur by lateral association of activated integrin receptors. Using supported bilayers with fluid, lipid-linked RGD ligands, large clusters of activated integrins formed in bound mouse embryo fibroblasts (Yu et al., 2011. PNAS 108:20585). After cells spread on RGD bilayers for 15 minutes, integrin clusters were formed even in the presence of inhibitors of cytoskeletal assembly. We have quantified the cluster size, geometry and receptor distribution at a nanometer level using photoactivated light microscopy. When the temperature was decreased to 250C, the cluster formation was greatly diminished, indicating that the integrin cluster formation may be an entropically driven process. To identify the players crucial for this process, we observed that when Talin1 knockout cells spread, the cluster size and the position of the clusters around the cell was altered. This was rescued by a full length Talin construct, indicating that Talin is required for the early cluster formation. This was also rescued by Talin head domain and not by Talin rod domain, indicating that most likely in the absence of external force, the integrin activation by Talin head was required for cluster to form. This study shows that the integrin cluster formation proceeds in the absence of external traction force in a Talin1 dependent manner. Thus, we suggest that talin head binding is an important factor in integrin clustering.

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Allosteric Regulation by Components of a Critical Membrane

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We present a simple model of a membrane bound protein allosterically regulated by the local lipid composition. We are motivated by the experimental finding that the plasma membranes of mammalian cells are tuned close to a liquid-liquid critical point, where the sensitivity of many properties to perturbations is large. We consider a protein whose boundary conditions with the surrounding membrane are dependent on its functional state (i.e. conducting vs. non-conducting for an ion channel). For such a protein we show that small changes in the chemical potential of lipids can lead to dramatic functional changes near a critical point. This type of regulation becomes more potent as the protein becomes larger, and as the membrane gets closer to a critical point. Such a protein would also have its nanometer-scale localization correlated with its functional state. A cell could regulate such a protein by adjusting the composition either by changing the ratio of ordered to disordered lipids (experimentally probed by cholesterol depletion and loading) or by raising or lowering the critical temperature. Here we focus on perturbations that act to lower the critical temperature, like the liquid general anesthetics that have been shown to lower critical temperatures by ~4K at clinically relevant concentrations. We show that this change is sufficient to lead to changes in channel conductivity in line with what has been shown for a wide class of channels even without specific interactions between perturbing molecules and the channel itself.

2644-Pos Board B336

Excitability of Guanylate Cyclase Signaling Pathway Mediating Chemotaxis

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Chemotaxis, the directed migration of cells in chemical gradients, is a vital function in many biological processes, for example, morphogenesis and synapse formation. In chemotaxis of many eukaryotic cells, the localization of some key signaling mediators on the cell membrane regulates cell movements. Chemotaxis of Dictyostelium cells is mediated by some parallel signaling pathways like phosphoinositide-3-kinase (PI3K) pathway, soluble guanylate cyclase(sGC) pathway and so on. Previous work demonstrated that the response to chemoattractant, cyclic adenosine monophosphate (cAMP), of PI3K pathway has some characteristics of an excitable system. The theoretical model shows that this excitable pathway can regulate cell unidirectional movements in respond to a chemical gradient efficiently. However, what kind of property sGC pathway has remains to be clarified and it also remains unknown about the interaction between the sGC pathway and the PI3K pathway.

Here, we studied that the sGC pathway also is an excitable system by analyzing the spatiotemporal dynamics of sGC. We found that focal sGC-enriched domains were spontaneously generated in a no external stimulus condition. The sGC-enriched domain generated spontaneously had same property with the domains induced by cAMP stimulation. These features are characteristics of an excitable system like the PI3K pathway. We will report that the dynamics of the PI3K pathway is influenced by modulation of the sGC pathway.

The chemotactic signal transduction consists of multiple excitable pathways. This structure of signaling system may increase efficiency of respond to the steep gradient of cAMP.

2645-Pos Board B337

Investigating Phosphatidylinositol 3,4-Bisphosphate 3-Phosphatase Activity of Ci-VSP in Xenopus Laevis Oocytes and Cho Cells using Fluorescent Phosphoinositide Probes

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The voltage sensitive phosphatase Ci-VSP is generally considered to be a $PI(4,5)P_2/PI(3,4,5)P_3$-5-phosphatase *in-vivo*, while 3-phosphatase activity could be demonstrated *in-vitro* using the isolated catalytic domain of Ci-VSP. However, a recent study demonstrates $PI(3,4)P_2$-3-phosphatase activity of Ci-VSP expressed in *Xenopus laevis* oocytes (Kurokawa et al, 2012, PNAS). This is in contrast to our previous findings obtained in Chinese hamster ovary (CHO) cells (Halaszovich et al., 2009, JBC).

We set out to do a careful re-evaluation of Ci-VSP's specificity in oocytes as well as CHO cells. TAPP1-PH-GFP was used as the $PI(3,4)P_2$ sensor. In some experiments, the resting level of PI-3-phosphates was increased using either co-expression of a constitutively active PI-3-kinase or stimulation of endogenous IGF-receptors with insulin. Membrane binding of TAPP1-PH-GFP as a measure of $[PI(3,4)P_2]$ was quantified using fluorescence microscopy (confocal LSM for oocytes, TIRF microscopy for CHO cells). The membrane voltage of oocytes was controlled using the two-electrode voltage-clamp technique, for CHO cells the patch-clamp technique was used.

We failed to demonstrate 3-phosphatase activity against $PI(3,4)P_2$ in oocytes but always detected production of this phosphoinositide species, which presumably reflects $PI(3,4,5)P_3$-5-phosphatase activity of heterologously expressed Ci-VSP as well as endogenous VSPs. However, in Ci-VSP expressing CHO cells we could detect a reduced increase, but not a decrease in TAPP1-PH-GFP binding to the cell membrane at high voltages. This reduction probably reflects $PI(3,4)P_2$-3-phosphatase activity and not reduced $PI(3,4,5)P_3$-5-phosphatase activity.

In conclusion, our findings show an overall production of $PI(3,4)P_2$ over the whole voltage range studied. Therefore we presume that under physiological conditions the $PI(3,4,5)P_3$-5-phosphatase activity of Ci-VSP outweighs its $PI(3,4)P_2$-3-phosphatase activity.

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2646-Pos Board B338

In-Situ Description of the Role of $PtdIns(3,4,5)P_3$ and PtdSer on PDK1 Regulation in Human Cancer Cells by Advanced Quantitative Microscopy

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3'-phosphoinositide dependent kinase-1 (PDK1) plays a central role in the transduction of signals downstream phosphoinositide 3-kinase (PI3K), a pathway often found deregulated in tumours. PDK1 regulation is known to respond to phosphatidylinositol(3, 4, 5)trisphosphate ($PtdIns(3,4,5)P_3$) levels and it has been recently shown that its PH domain specifically binds to